ICES Journal of Marine Science, 56: 449–458. 1999
Article No. jmsc.1999.0452, available online at http://www.idealibrary.com on IDEAL®

The evolutionary history of steelhead (*Oncorhynchus mykiss*) along the US Pacific Coast: Developing a conservation strategy using genetic diversity

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Nielsen, J. L. 1999. The evolutionary history of steelhead (*Oncorhynchus mykiss*) along the US Pacific Coast: Developing a conservation strategy using genetic diversity. – ICES Journal of Marine Science, 56: 449–458.

Changes in genetic variation across a species range may indicate patterns of population structure resulting from past ecological and demographic events that are otherwise difficult to infer and thus provide insight into evolutionary development. Genetic data is used, drawn from 11 microsatellite loci amplified from anadromous steelhead (*Oncorhynchus mykiss*) sampled throughout its range in the eastern Pacific Ocean, to explore population structure at the southern edge in California. Steelhead populations in this region represent less than 10% of their reported historic abundance and survive in very small populations found in fragmented habitats. Genetic data derived from three independent molecular systems (allozymes, mtDNA, and microsatellites) have shown that the southernmost populations are characterized by a relatively high genetic diversity. Two hypothetical models supporting genetic population substructure such as observed were considered: (1) range expansion with founder-flush effects and subsequent population decline; (2) a second Pleistocene radiation from the Gulf of California. Using genetic and climatic data, a second Pleistocene refugium contributing to a southern ecotone seems more feasible. These data support strong conservation measures based on genetic diversity be developed to ensure the survival of this uniquely diverse gene pool.

Key words: conservation, evolution, genetic diversity, microsatellite DNA, steelhead.

Received 27 March 1998; accepted 27 October 1998.

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Introduction

Conservation and recovery strategies for threatened or endangered aquatic species are usually developed using demographic and/or ecological (habitat based) data. These data typically derive from surveys of a small number of populations over a short period of time. Concordance of results drawn from demographic and ecological data is not guaranteed. Extinction risk models used to analyse population trends at the local and global scales consider three kinds of stochastic change: environmental, demographic, and catastrophic (Mace and Lande, 1991; Boyce, 1992; Caughley, 1994; Taylor, 1995), but neglect potential information derived from genetic effects (Dunham *et al*., 1999). Environmental and catastrophic change affect all individuals within a population and can have large negative results on genetic population structure. Demographic change, on the other hand, affects each individual differently and can result in multiple dispensations of fitness among individuals within the population.

Population decline, quasi-extinction, and low overall abundance may result from any of the above effects, but genetic structure within and among survivors will differ. A catastrophic loss of most individuals within a population in a given area can lead to recolonization by neighbour populations with similar ecological and reproductive requirements. Colonizers, however, can quickly swamp most of the original gene pool. Expansion of a species range due to environmental shifts in habitat conditions or invasions following catastrophic change can retain significant genetic structure with sufficient gene flow, and theoretically lead to unique patterns of increased genetic diversity due to founder-flush

1054–3139/99/040449+10 $30.00/0

events (Slatkin, 1996). On the other hand, altered environmental conditions that accompany demographic expansion may shift fitness and survival patterns within a population and can have a variety of effects on the gene pool. Changes in effective population size (the number of individuals actually contributing genes to the next generation), population bottlenecks, and movement (i.e. gene flow) among surviving groups must be considered (Avise, 1994; Allendorf and Waples, 1996).

Under all of the above conditions, the degree of genetic variation found among and between existing populations provides information concerning past patterns of demographic and ecological events in the history of each population (Weir, 1996). Patterns of genetic variation have a strong historical component (Slatkin, 1985, 1993) and trends in isolation, gene flow, and evolutionary history derived from these may provide insight into historic population dynamics (Avise, 1994). These changes are especially informative in fragmented populations or those found near the edge of a species range. The suite of events reflected in the gene pool can be used in the analyses of population viability or extinction risk (Lande, 1995; Hedrick *et al*., 1996; Allendorf *et al*., 1997). Therefore, genetic data can serve as indicators of conservation and recovery requirements that are otherwise difficult to infer.

Genetic diversity can be measured on multiple scales. Evolutionary divergence is a dynamic process with markers acting across many different temporal and spatial patterns (Avise, 1994; Nielsen *et al*., 1997a). Changes in molecular markers used in population studies are generally thought to be selectively neutral throughout their evolutionary range and have no direct effect on individual fitness (Kimura, 1983). Change is assumed to result from stochastic events involving differences in genetic drift and/or gene flow among the different populations (Nei, 1987). Neutrality, therefore, is the most parsimonious explanation for patterns of genetic variation found in fish population studies.

DNA mutation rates are known to vary extensively among genes and across taxa (Wilson *et al*., 1977; Nei, 1987; Weber and Wong, 1993). With highly variable mutation rates and no evidence of selection, the three most common molecular systems—allozymes, mitochondrial DNA (mtDNA), and microsatellite repeat loci—can present three different temporal or spatial scales for measuring genetic diversity in any population (Boyce *et al*., 1996; Nielsen *et al*., 1997b). Allozyme electrophoresis reflects protein-coding genetic structure and loci in this system are thought to have the slowest mutation rate among the three markers. Allozyme analysis was the only molecular system applied to fish population studies until the early 1980s when the development of DNA technology provided new tools (Utter, 1995).

Mitochondrial DNA (mtDNA), passed from the female of the species to her offspring, has been shown to provide strong biogeographic resolution in many species (Avise, 1994). Maternally inherited, non-recombining mtDNA markers with high evolutionary rates of sequence divergence can show a degree of variation among populations not found in more slowly evolving proteins and enzymes (Brown *et al*., 1979; Moritz *et al*., 1987).

Microsatellite DNA represents a class of highly polymorphic, simple sequence tandem repeat loci, usually containing two (di-), three (tri-), or four (tetranucleotide) core units. These units repeat at varying lengths in different individuals, groups, or populations. Microsatellites often have more alleles per locus by species than allozymes and are more variable than haplotype diversity depicted by mtDNA. These markers exhibit high levels of variability due to high mutation rates (10^{-3}–10^{-4}) and the large numbers of unlinked loci available from most animal genomes (e.g. Queller *et al*., 1994). Additionally, microsatellites can be amplified from non-destructive tissue and changes recorded in the loci are frequently used to determine genetic variation in studies of closely related or endangered species (Ashley and Dow, 1994; May *et al*., 1997). With molecular changes occurring at different mutation rates in different markers, congruence among these markers would require some degree of consistency in population history and structure across multiple temporal and/or spatial scales (Boyce *et al*., 1996).

Under the ecological "dispersal" model, populations found at the edge of their species' range are thought to be made up of colonizers from the centre of the range that have adapted to new environmental conditions through active or passive dispersal across a pre-existing geographic or ecological barrier (Avise, 1994). In the alternative model of "vicariance" a more or less continuous distribution of a species across the landscape has been split by large-scale geologic or hydrologic events such as uplifting, continental break-up, or glaciation. In this study I examine microsatellite genetic diversity for 11 loci among four California anadromous steelhead (*Oncorhynchus mykiss*) populations and one population from southeast Alaska. Genetic diversity analyses are used to examine the population structure of steelhead at the southern extent of their range, and to gain insight into their past evolutionary history with consideration of two very different genetic dispersal models. Microsatellite diversity measures from several aquatic and terrestrial species from the published literature are compiled for comparison with diversity measures developed from Pacific steelhead.

Material and methods

Individual fish used in this paper were collected for various projects, 1989–1997 (Table 1). Southern steelhead were represented by 30 fish captured in the Santa

Table 1. Anadromous steelhead populations sampled for microsatellite genetic analyses.

Region	Population	Year(s)	N
California:			
South of Point Conception	Malibu Creek	1993–1994	14
North of Point Conception	Santa Ynez River	1993–1994	16
South of Cape Mendocino	Albion River	1993	3
	Cottonova Creek	1992	2
	Garcia River	1992	3
	Gualala River	1991	4
	Howard Creek	1992	3
	Navarro River	1992	4
	Usal Creek	1991	3
	Van Duzen River	1993	5
North of Cape Mendocino	Middle Fork River	1992–1993	11
Southeast Alaska	Sashin Creek	1996	59

Ynez River (36 km north) and Malibu Creek (96 km south) near the Point Conception species boundary in southern California (Figure 2). These fish represented the total observed anadromous contribution (spawning year 1993/1994) for the southern most populations in the Eastern Pacific Ocean. Northern California steelhead (n=27) were collected from eight coastal rivers found south of Cape Mendocino. Middle Fork Eel River fish were collected from winter-run steelhead (summer-run fish also occur in this basin). The Eel River flows into the Pacific Ocean approximately 200 km north of Cape Mendocino and 150 km south of the Oregon border with California. Southeast Alaska steelhead were collected by the National Marine Fisheries Service in the Sashin Creek drainage on the southeast portion of the Baranof Island near Little Port Walter.

Previously published data for California steelhead based on allozymes (Busby *et al*., 1996) and mtDNA (Nielsen *et al*., 1994; Nielsen *et al*., 1998) provide protocols and methodologies for these two molecular systems. New genetic data presented here are based on amplification of 11 microsatellite loci (Omy77, Omy207, Omy325, Oneμ2, Oneμ8, Oneμ11, Oneμ14, Ots1, Ssa14, Ssa85, and Ssa289) drawn from other research reports and publications. My laboratory has previously reported data on Omy77, Omy207, and Ssa289 for California steelhead. Our microsatellite DNA extraction and amplification protocols are available in this literature (Nielsen *et al*., 1997a).

The 11 loci reported here have shown significant polymorphisms in previous studies across a broad range of salmonid species. The Omy-series of microsatellite loci were developed specifically for *O. mykiss* (Morris *et al*., 1996; M. O'Connell, Dalhousie University, pers. comm.), the Oneμ-series was developed for *O. nerka* (Scribner *et al*., 1996), Ots-series for *O. tshawytascha* (M. Banks, University of California, Davis, pers. comm.), and the Ssa-series of primers were developed for Atlantic salmon, *Salmo salar* (McConnell *et al*., 1995; O'Reilly *et al*., 1996).

The underlying molecular mechanisms of the mutation process remain unknown for microsatellites leading to controversy in analytical assumptions used to measure genetic diversity and differentiation. For this study, I ran two pairwise genetic distance matrices based on the current most popular mutation models. The first was calculated for microsatellite allelic diversity using methods described in Goldstein *et al*. (1995) for the 11 microsatellite loci combined. Their $(\delta\mu)^2$ genetic distance measure is equivalent to a general analysis of variance using an average sum of squares of differences in allelic size within each population, and the average squared difference between all possible pairs of populations. These calculations maintain an expectation of mutation events under a strict, single-step (± one repeat unit) shift for each event.

Nei's genetic distance D based on a similar computational approach, but using the infinite allele model of mutation (Nei, 1972; Nei *et al*., 1983), was also calculated among the five Pacific steelhead populations using data from the 11 microsatellite loci combined. The infinite allele model incorporates no expectation of correlation between allele size and the number of mutation events. Both distance measures represent a scale of variation for allelic frequencies among and between populations and were calculated using the program (MICROSAT 1.4) available from Dr E. Minch, Department of Genetics, Stanford University, USA (http://lotka.stanford.edu/distance.html).

Distance data $(\delta\mu)^2$ were used to generate an unrooted consensus neighbour-joining tree using the NEIGHBOR81 and CONSENSE applications from PHYLIP (Felsenstein, 1993) comparing microsatellite diversity among the five geographically divergent steelhead populations. One-thousand replicate microsatellite distance trees were generated to obtain bootstrap estimates and

Table 2. Allelic size distributions found in 11 microsatellite loci for six anadromous steelhead populations (cf. Table 1; SEAA: southeast Alaska; PS: Puget Sound, Washington state; MFER: Middle Fork Eel River; NCA: northern California south of Cape Mendocino; SYR: Santa Ynez River; MC: Malibu Creek). n: number of fish sampled. Numbers in parentheses represent positive observations of the number of alleles per locus.

		Population					
		SEA	PS*	MFER	NCA	SYR	MC
Locus	n	59	51–58	11	27	16	14
Omy77		103–137 (9)	117–137 (11)	99–143 (9)	93–147 (14)	97–141 (15)	103–151 (8)
Omy207		102–138 (10)	n.a.	102–142 (7)	102–146 (11)	104–154 (10)	104–156 (10)
Omy325		95–135 (11)	95–151 (20)	87–127 (13)	99–121 (11)	101–131 (10)	103–131 (9)
Oneμ2		204–284 (22)	237–350 (23)	224–270 (14)	204–270 (15)	206–240 (8)	224–238 (5)
Oneμ8		159–173 (3)	150–178 (9)	145–173 (7)	145–171 (6)	157–169 (4)	157–159 (2)
Oneμ11		143–149 (4)	144–146 (2)	145–147 (2)	143–149 (4)	145–149 (3)	141–147 (3)
Oneμ14		147–171 (9)	147–163 (4)	147–171 (5)	147–161 (5)	151–157 (4)	151–157 (4)
Ots1		159–239 (9)	159–251 (10)	159–165 (3)	159–171 (6)	155–183 (7)	159–241 (5)
Ssa14		130–154 (8)	129–155 (10)	136–166 (8)	130–158 (11)	134–152 (9)	126–152 (5)
Ssa85		105–137 (7)	105–135 (8)	105–149 (5)	105–141 (5)	99–131 (5)	105–115 (2)
Ssa289		110–124 (5)	n.a.	110–124 (7)	110–124 (8)	110–124 (6)	110–120 (6)

*From Wenburg *et al.*, 1996.

assess reproducibility of branching patterns found in the consensus tree.

A measure of the effects of population subdivision based on a variance-ratio test commonly used in population genetic studies (Fst) was calculated for 11 microsatellite loci combined using MICROSAT. MICROSAT was also used to estimate the extent of gene flow (M) in an island model at equilibrium (Slatkin, 1993) among all possible pairs of steelhead populations. Geographic distance between any two populations was calculated as coastal kilometres between the centre of the geographic distribution of each collection. We tested for a pattern of isolation by distance among the five steelhead populations by regressing $^{10}\log(M)$ on $^{10}\log(\text{distance})$ according to methods given in Slatkin (1993). Based on simulation and tests of empirical data, Slatkin (1993) showed that a negative slope resulting from this type of regression analysis would suggest some degree of isolation by distance among equilibrium populations.

Results

Analyses of steelhead genetics used 11 microsatellite loci, 9 of which have published allelic ranges for steelhead populations from Washington state (Wenburg *et al.*, 1996). Allelic size (including the amplified primer) ranged from 87 to 320 base pairs (bp). Allelic size ranges presented here exceeded (high or low) previously published ranges for all 9 loci (Table 2). For all populations combined, the number of alleles per locus averaged 16, and ranged from 4 (Oneμ11) to 35 (Oneμ2). Average heterozygosity for the 11 loci was 0.68. Average Fst for these loci in the five steelhead populations used in this study was 0.17.

We found 23 unique alleles in the southern steelhead samples from the Santa Ynez River and Malibu Creek. The average frequency of four of these alleles exceeded 0.05 (traditional cut-off for the definition of "rare alleles" in population genetics) in the combined southern steelhead collection. At the Oneμ2 locus we found a group of 24 alleles ranging in size from 242 to 320 bp, that were common in both northern California and Alaska steelhead, but were never found in southern stocks. This large size range expansion of alleles for this locus suggests a possible duplication event in steelhead evolutionary history similar to the many duplicated allozyme loci thought to be related to the tetraploid ancestry of all salmonids (Allendorf and Thorgaard, 1984).

Genetic distance analysis based on allelic structure at the 11 loci combined showed the greatest genetic distance between southern (from the Santa Ynez River and Malibu Creek) and southeast Alaska steelhead [$(\delta\mu)^2=51.17$; $D=1.18$]. In comparison, genetic distance between steelhead from the Middle Fork Eel River and northern California populations found south of Cape Medocino was only $(\delta\mu)^2=2.68$ ($D=0.094$). Genetic distance between the Alaska steelhead and those found in California rivers south of Cape Mendocino was $(\delta\mu)^2=21.72$ ($D=0.77$). Neighbour joining analysis of the $(\delta\mu)^2$ genetic distance data supported separation of the southern steelhead populations from all other groups with a bootstrap value of 57% and separation between Santa Ynez River fish and those collected in Malibu Creek at 73% (Figure 1).

Estimates of Fst based on microsatellite allelic frequencies in our five steelhead collections were all less than 10%. Fst for California steelhead ranged from Fst=0.03 (Malibu Creek) to Fst=0.05 (Middle Fork Eel

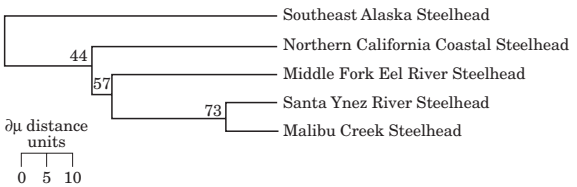

Figure 1. Consensus neighbour-joining tree for 11 microsatellite loci based on $(\delta\mu)^2$ genetic distance measures calculated for five populations of anadromous steelhead found throughout their range in the eastern Pacific Ocean. Bootstrap values are given as the percentage of trees out of 1000 that support branching patterns to the right of the bootstrap numbers.

River). Fst calculated for southeast Alaska steelhead from Sashin Creek was 0.05. Estimates of gene flow (Slatkin's M) ranged from 4.75 (southeast Alaska and Malibu Creek) to 10.16 (south of Cape Mendocino and Middle Fork Eel River populations). Regression analysis to test for isolation by distance among the five populations had a slope of -0.11 with $r^2=0.12$. This analysis improved support for isolation by distance when we analysed northern California and Alaska populations independent of southern California fish, giving a slope of -0.28 and $r^2=0.34$.

Discussion

Several genetic analyses of coastal steelhead have been published previously. Sequence analyses of 188 bases of the mtDNA control-region adjacent to the phenylalanine tRNA gene showed a distinct biogeographic cline in mtDNA haplotypes along coastal California (Figure 2; Nielsen *et al*., 1994). Allozyme analyses by the National Marine Fisheries Service of some of the same populations of southern steelhead we used for this study showed unprecedented levels of allelic diversity in southern stocks (Busby *et al*., 1996).

Control-region mtDNA sequence for southern steelhead in California showed haplotype diversity=0.57 and nucleotide diversity=0.014 (Nielsen *et al*., 1997a; Nielsen *et al*., 1998). We can compare these values to other published diversity estimates for mtDNA sequence data in fish: nucleotide diversity in California rainbow trout=0.0034 (Bagley, 1997); chinook (*O. tshawytscha*) haplotype diversity ranged from 0.17 to 0.48 and nucleotide diversity from 0.0019 to 0.0044 (Adams *et al*., 1994); North American whitefish (*Coregonus* sp.) nucleotide diversity=0.0007 (Bernatchez and Dodson, 1994); haplotype diversity in walleye (*Stizostedion vitreum*)=0.36–0.79 (Stepien and Faber, 1998). In these comparisons only walleye showed significantly more diversity for mtDNA sequence data. However, the latter were based on an analysis of larger segments of mtDNA including variable sections containing tandem repeats found at the proline tRNA end of the control-region in walleye.

Our current analyses using 11 microsatellite loci showed unique genetic diversity in southern California steelhead stocks. Recent studies of genetic distance among diverse taxa suggested that $(\delta\mu)^2$ and D are efficient measures of tree branch length estimates and can be used to reconstruct an evolutionary topology among taxa (Nei, 1995; Takezaki and Nei, 1996). Both measures present relevant scales for understanding genetic relationships among geographically divergent steelhead populations. Available publications on microsatellite diversity allow a comparison with those found in other fish and mammal populations (Table 3).

The genetic distance measures calculated between southern California and Alaska steelhead was significantly higher than within basin comparisons for Alaska steelhead and California rainbow trout in the Sacramento River. Only an analysis of five North Atlantic cod populations produced similar $(\delta\mu)^2$ values greater than 50. Our estimate of Nei's genetic distance (D) was exceeded only in a comparison of chimpanzees and humans, and in two papers looking at wild and domestic bighorn sheep. The reported values for Atlantic salmon are slightly lower, whereas chinook salmon has apparently a very low D.

In the face of their extensive genetic diversity, California steelhead numbers have declined significantly over the last century. Of the populations in 122 streams south of San Francisco that had documented steelhead runs, 27% are now extinct and the remaining populations contain less than 10% of their historic abundance (McEwan and Jackson, 1996). Declines are most severe in the most southern populations. The combination of very low effective population size and very high genetic diversity appears counter intuitive and goes against most common molecular evolution models where the greatest diversity is expected to exist in populations with the largest number of breeders.

Several hypotheses have been suggested as the cause for unique genetic diversity found at the edge of this species' range. One hypothesis following the "dispersal" model cites climatic cycles in ocean conditions lasting 40–200 years which can cause a shift in abundance of anadromous salmon and steelhead among habitats throughout the eastern Pacific Ocean (Pearcy *et al*., 1990; Pearcy, 1992). Under this hypothesis, as the cycles repeat themselves there are periodic or episodic opportunities for significant demographic and genetic exchange among populations. As favourable ocean conditions return, salmon and steelhead stocks could recolonize southern habitats with increased survival (Lawson, 1993). But steelhead have shown significant declines coast wide since the 1980s (Busby *et al*., 1996), not just in southern California.

Cyclic change in ocean conditions could have theoretically populated the southern California coastal streams with fish from diverse lineage's breeding in more

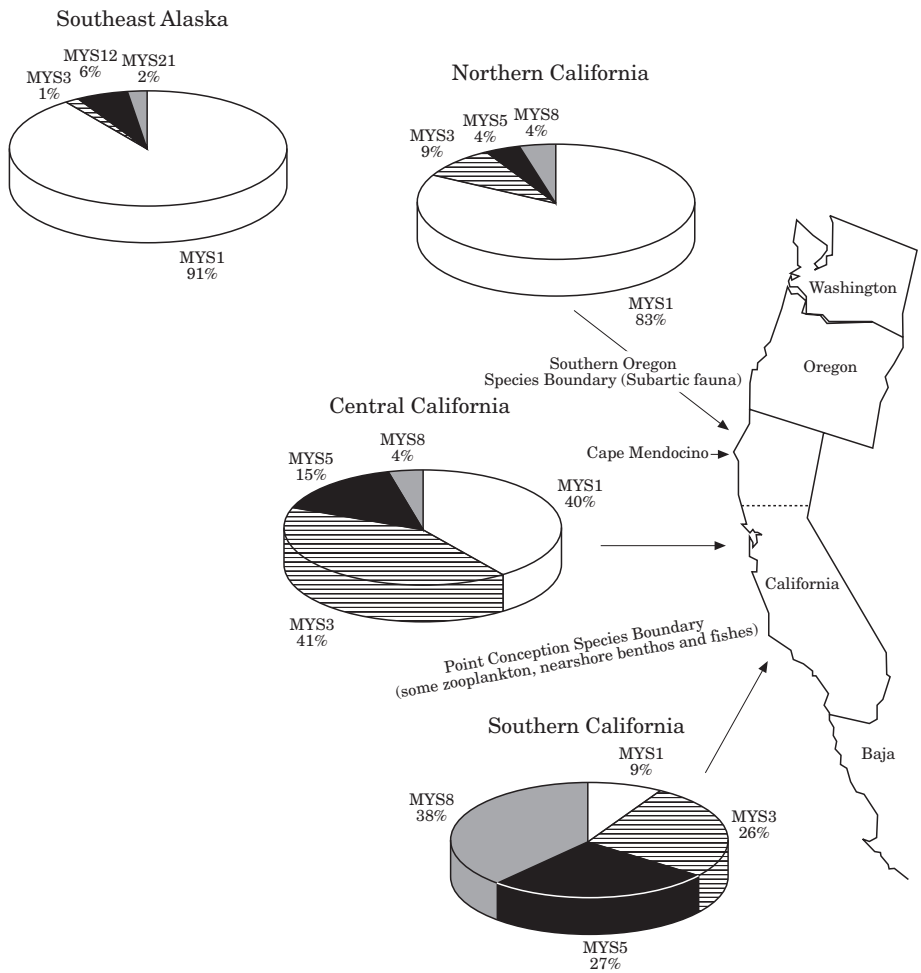

Figure 2. Mitochondrial DNA haplotype frequency distributions in three bio-regions of California and from southeast Alaska. Two transition zones or species boundaries are shown, one near Point Conception, and the subarctic boundary near Cape Blanco in southern Oregon. MYS5 and MYS8 were only found in California populations; MYS12 and MYS21 represent the third and fourth most common haplotypes in southeast Alaska populations. Numbers of individuals sampled exceed 80 per region (for details see Nielsen *et al*., 1994 and Nielsen, 1996).

northern environments. Much of the unique genetic diversity found for allozymes, mtDNA, and microsatellites in southern steelhead has not been found at all in contemporary Pacific steelhead collections from more northern areas, leading to questions about where parental populations for the southern steelhead were located. Congruence among the three independent genetic markers for unique diversity suggests that past events leading to genetic structure in these populations were consistent with population history and structure over a large range of temporal scales. Otherwise, independent molecular systems with very different mutation rates would show unique scales of diversity without any expectation of congruence (Boyce *et al*., 1996).

Common alleles found in the northern California populations are also found in southern areas supporting a hypothesis of significant gene flow between these areas. Our analyses indicated a limited pattern of isolation by distance with a slope of -0.11 in the regression of Slatkin's M (gene flow) on geographic distance among our study populations. This analysis, however, showed that only 12% of the variation found in the microsatellite data could be explained by isolation by distance. In a similar analysis using 24 allozyme loci in gulls (*Larus glaucescens*) collected along the west coast of North America, isolation by distance explained 37% of the variance (Slatkin, 1993).

Low regression slope and r^2 values found for comparisons using southern steelhead could result from two factors. Either there is significant geneflow among these populations and very little actual isolation by distance, or one or more population is not in genetic equilibrium.

Table 3. A comparison of molecular diversity measures calculated using microsatellites. Slatkin (1993) isolation by distance measure calculated from Fst is given as M. Genetic distance values based on $(\delta\mu)^2$ were derived from Goldstein *et al.* (1995). Nei's distance (1972) is given as D. Maximum distance values are those recorded in source population comparisons (see individual papers for exact population distributions). Hz=heterozygosity.

Population	n	# loci	Average Hz	Mean Fst	M	Max. $(\delta\mu)^2$	Max. D	Source
Fish								
Between southern California and Alaska steelhead	89	11	0.66	0.029	8.37	51.17	1.182	This paper
Between Alaska and northern California steelhead	86	11	0.71	0.025	7.56	21.71	0.772	This paper
Between southern and northern California steelhead	57	11	0.72	0.064	10.17	12.52	0.249	This paper
Between Mendocino coast and M. F. Eel River steelhead	38	11	0.72	0.035	6.69	2.68	0.094	This paper
Among 11 populations Sacramento R. rainbow trout	732	12	0.68	0.018	13.64	8.36	0.182	Nielsen unpub. data
Among 3 populations Alaska rainbow trout/steelhead	59	10	0.46	0.087	2.62	13.22	0.289	Nielsen unpub. data
Among 3 populations chinook salmon Yukon River	83	16	0.27	0.053	4.47	0.33	0.084	Scribner *et al.*, 1996
Among 7 Atlantic salmon populations in Quebec	183	5	0.83	0.075	3.08	n.a.	0.897	Fountain *et al.*, 1997
Between Canadian and north Ireland Atlantic salmon	120	3	0.53	n.a.	n.a.	n.a.	0.900	McConnell *et al.*, 1995
Among 5 populations North Atlantic cod	101	6	0.90	0.014	17.61	50.60	n.a.	Bentzen *et al.*, 1996
Mammals								
Among 8 international human populations	318	8	0.63	0.107	2.09	n.a.	0.346	Deka *et al.*, 1995
Between Navajo Native Americans and Finnish Europeans	402	33	0.67	n.a.	n.a.	n.a.	0.399	Urbanek *et al.*, 1966
Among 14 indigenous human populations	140	25	0.57	0.163	1.29	25*	0.548	Bowcock *et al.*, 1994
Comparing humans and chimpanzees	186	8	0.61	n.a.	n.a.	n.a.	1.901	Deka *et al.*, 1995
Among 1 wild and 3 domestic bighorn sheep populations	250	8	0.77	0.155	1.36	39*	2.779	Forbes *et al.*, 1995
Among bighorn sheep in 15 southwest populations	252	4	0.56	0.272	0.67	n.a.	1.283	Boyce *et al.*, 1996

*Distance values taken from estimates made in Takezaki and Nei (1996).

A second example given by Slatkin (1993) concerned pocket gophers (*Thomomys bottae*) in central California where he concluded that the time scale of approach of Fst, and hence M, to equilibrium (where isolation by distance becomes apparent) is under the influence of local effective population size. A recent immigration event (past 50–2000 years) forced him to reject a pattern of isolation by distance. A similar conclusion was reached for these populations using genetic distance (Lessa, 1990).

A population is in genetic equilibrium when there are more or less constant allele rations in a gene pool through successive generations over a significant period of time. The number of rare alleles found to be unique to the very small southern steelhead populations argue against equilibrium. Analysis of northern California and Alaska steelhead without southern California stocks produced slope (-0.28) and r^2 (0.34) values similar to those reported as significant in Slatkin (1993). These results suggest the northern California populations may fit the assumptions of equilibrium and do show significant isolation by distance, but southern stocks do not.

Founder-flush effects represent one theoretical condition under which extensive genetic diversity can evolve rapidly from a single, panmictic source, although the theory remains controversial (Templeton, 1980; Slatkin, 1996; Charlesworth, 1997). Founder-flush models assumed that populations that are founded by a small number of individuals, and then grow rapidly, are characterized by a distinct genetic evolution. If the founding population represents a random sample from the parent population, the model predicts that during the period of rapid growth, genetic drift is much weaker than would normally be expected, particularly for low-frequency alleles. Therefore, the probability of loss of a neutral lineage is low and the probability of fixation of advantageous alleles is much higher than in populations that remain a constant size (Slatkin, 1996).

For founder-flush effects to have contributed to the observed genetic diversity we would need to find support for range expansion into the southern habitats, a period of rapid growth, and a period of habitat reduction (ocean and fresh water) leading to a significant and rapid population decline. Range expansion and habitat decline seem to be feasible circumstances in the recent evolutionary history of southern California steelhead. While ecological conditions in this region during the Pleistocene are not well documented, the extreme temperatures and arid environment found in today's freshwater habitats aruge strongly against rapid population growth over recent geologic history.

Putting aside the founder-flush hypothesis due to lack of supporting evidence, we are left with one alternative to explain the unique genetic diversity observed. This second hypothesis follows the "vicariance" model of genetic variation outlined in Avise (1994). Behnke (1992) suggested a Gulf of California Pleistocene refugium for Pacific salmonids. Perhaps some of the genetic diversity in southern steelhead represents lineage effects from populations that evolved from a Gulf of California refugium, rather than reflecting particular processes in a marginal population with common ancestry from a Beringia refugium. If this is true, then current populations would be located close to the centre of their Pleistocene divergence and not at the southern edge. A Gulf of California refugium has been documented for many marine and nearshore organisms (Lynn and Simpson, 1987; Thomas and Strub, 1990; Bernard *et al.*, 1991).

This hypothesis suggests that southern California may play the role of an evolutionary ecotone (after Smith *et al.*, 1997) between two Pleistocene source populations. Interfacing divergent conditions found in ecotones play a significant role in generating genetic diversity (Mace *et al.*, 1996). Small isolated populations found in such regions may exhibit greater interpopulation diversity because individuals are more subject to drift and/or directional selection (Smith, 1993; Risser, 1995).

Physical ocean conditions support the ecotone hypothesis. The California Transition Zone (equivalent to the southern species boundary in Figure 2) represents a region of steep thermal gradients situated adjacent to Point Conception, California (34°N). It has been shown that boundaries of distributions of individual species will cluster in regions of pronounced environmental change (Bernard *et al.*, 1991). This may also be true for populations of steelhead that carry variable biogeographic histories, having evolved from two divergent Pleistocene refugia (Nielsen *et al.*, 1994).

If the theory of two Pleistocene refugia and an ecotone in southern California where the two evolutionary histories meet and combine is true, it has important implications for management, because differing processes or pathways of evolution need to be considered in conservation programmes (Smith *et al.*, 1993). The National Marine Fisheries Service has recently determined steelhead in southern California as an Evolutionarily Significant Unit (ESU) of *O. mykiss* and listed it as an endangered "species" under the US Endangered Species Act. Although some portion of their genetic diversity has survived, extinction of this unique gene pool is highly likely unless conditions in freshwater habitats improve (Busby *et al.*, 1996). Recolonization and/or restoration of these areas by Beringia derived fish (i.e. northern stocks) would not adequately reflect their existing genetic diversity. Unique evolutionary histories found in any population provide a mandate for careful and considered recovery activities (Nielsen, 1995; Tessier *et al.*, 1995; Allendorf and Waples, 1996). This is clearly the case for the southern steelhead in California.

Acknowledgements

I want to thank the many individuals who have helped with parallel studies of *O. mykiss* that contributed to this analysis. Special thanks go to B. Behnke, M. Fountain, C. Gan, M. Golden, R. Lande, S. McKinnell, M. Slatkin, F. Thrower, and D. Powers. Early versions of this paper were greatly improved by reviews from N. Daan, R. Waples, and F. Thrower. Partial funding for this study was provided by the Pacific Southwest Research Station, US Forest Service, NOAA National Marine Fisheries Service Staltonstall-Kennedy grant #NA76FD0047, and US National Marine Fisheries Service, Auke Bay Laboratory.

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